Journal of Molecular Diagnostics, Vol. 6, No. 3, August 2004

A Study of *MECT1-MAML2* in Mucoepidermoid Carcinoma and Warthin's Tumor of Salivary Glands

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The t(11;19)(q21;p13) chromosomal translocation has been described in two distinct types of salivary gland neoplasms: mucoepidermoid carcinoma (MEC) and Warthin's tumor (WT). Since this translocation has been recently shown to generate a *MECT1-MAML2* fusion gene, we evaluated 10 primary MEC and seven primary WT to further define the molecular association of these two entities using cytogenetic, as well as *in situ* hybridization (ISH) and reverse transcriptase-polymerase chain reaction (RT-PCR) analyses directed against the fusion gene. A karyotype was established in all neoplasms except for two MEC cases. Of the eight karyotyped MECs, five showed the t(11;19)(q21;p13), two had a normal karyotype, and one case presented a −Y and +X. Three of the WT revealed a normal karyotype and four had several abnormalities which did not involve chromosomes 11 and 19. ISH analysis performed in cytogenetic suspension and/or in tumor paraffin sections demonstrated *MAML2* rearrangement in 7 of 10 cases of MEC: all five cases with t(11;19), one case with normal karyotype, and one unkaryotyped case. RT-PCR analysis confirmed the expression of the *MECT1-MAML2* gene in all MEC cases that were positive by ISH analysis. Neither the t(11;19) nor *MECT1-MAML2* was detected in any case of WT, nor in control samples from polymorphous low-grade adenocarcinoma, acinic cell carcinoma, or normal parotid gland tissue. We have demonstrated that ISH and RT-PCR are sensitive methods for detecting *MECT1-MAML2* in MEC. In contrast, we did not detect the t(11;19) nor *MECT1-MAML2* expression in seven cases of WT. *(J Mol Diagn 2004, 6:205–210)*

Mucoepidermoid carcinoma (MEC) of the salivary glands represents 5% of all salivary gland tumors and 20% of the malignant forms.[1] To date, the karyotypic profile in this tumor type has only been described in 26 cases:[2] in seven cases there were rearrangements of 11q14–21 and 19p12–13, mainly as a chromosomal translocation t(11;19)(q21;p12–13)[3–7] and it was the sole abnormality in two cases.[4,7] The remaining five cases showed either a more complex translocation involving other chromosomes or other rearrangements.[3,5,6] The same abnormality has also been described in mucoepidermoid carcinomas originated in bronchial glands of the lung.[8,9] Interestingly, the t(11;19)(q13–21;p12–13) was also reported in Warthin's tumor (WT),[10,11] a benign salivary gland neoplasm, which suggested an unexpected cytogenetic association between two otherwise unrelated salivary gland tumors. However, it is known that WT can arise and/or co-exist with MEC,[12–14] warranting the reappraisal of this association.

The t(11;19)(q21;p13) found in MEC has recently been cloned.[15] Two genes are involved: *mucoepidermoid carcinoma translocated 1* (*MECT1*) gene and a member of the *mastermind-like gene family* (*MAML2*) located at 19p13 and 11q21, respectively.[15] This translocation generates a chimeric gene *MECT1-MAML2* that fuses exon 1 of *MECT1* with exons 2–5 of *MAML2*. *MECT1-MAML2* fusion product disrupts the normal mechanism of the Notch signaling pathway, activating Notch-target genes independently of exogenous signals, therefore representing a novel mechanism for altered Notch function in tumorigenesis.[15] In addition, the recent identification of the *MECT1* gene product as a potent co-activator for genes that are regulated by cyclic AMP responsive elements suggests that *MECT1-MAML2* may be disrupting both Notch and CREB signaling pathways to induce tumorigenesis.[16,17]

To further substantiate the importance of the *MECT1-MAML2* fusion gene in MEC tumorigenesis and to investigate a common molecular pathway with WT, we studied a series of 10 primary MEC and seven primary WT salivary gland tumors using conventional cytogenetics, *in situ* hybridization (ISH), and reverse transcriptase-polymerase chain reaction (RT-PCR).

Accepted for publication March 30, 2004.

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Table 1. Clinical, Cytogenetic, ISH, and RT-PCR Data from the Tumor Collective

Case	Sex/Age	Diagnosis	Location	Karyotype	Positive FISH	Positive ISH	Positive RT-PCR
1	F/61	MEC grade II	Parotid	46–47,XX,t(4;6)(p14;q21),**t(11;19)(q21;p13)**[cp5]	yes	yes	yes
2	M/58	MEC grade III	Minor	Complex, not established	no	nt	no
3	F/63	MEC oncocytic	Palate	46,XX[8]	nm	yes	yes
4	F/68	MEC grade I	Parotid	46,XX,**t(11;19)(q21;p13)**[19]	yes	nr	yes
5	M/78	MEC grade III	Parotid	45,X,−Y[3]/46–47,XXY[cp2]/46,XY[7]	nt	nt	no
6	F/74	MEC grade II	Parotid	46,XX,**t(11;19)(q21;p13)**[10]/46,XX[8]	yes	yes	yes
7	F/71	MEC grade II	Parotid	46,XX,**t(11;19)(q21;p13)**[4]/46,XX[4]	yes	yes	yes
8	M/39	MEC grade II	Parotid	46,XY[12]	nt	nt	no
9	F/15	MEC grade II	Palate	nm	nt	yes	yes
10*	F/29	MEC, ex-WT	Parotid	37–47,XX,der(11)del(11)(q21)**t(11;16;19)(q21;p13.3;p12 ~ 13)**, der(16)del(16)(p13.3)**t(11;16;19)(q21;p13.3;p12–13)**del(16)(q22), der(19)del(19)(p12 ~ 13)**t(11;16;19)(q21;p13.3;p12 ~ 13)**[20]	yes	nr	yes
11†	M/67	WT	Parotid	40–46,XY,add(3)(p26),del(11)(p13)[2]/46,XY[8]	nt	nt	no
12†	M/41	WT	Parotid	46,XY[22]	nt	nt	no
13†	M/62	WT	Parotid	46,XY[15]	nt	nt	no
14†	M/54	WT	Parotid	45–47,XY,t(6;15)(p21;q15)[5]/46,XY[7]	nt	nt	no
15†	M/68	WT	Parotid	46,XY[31]	nt	nt	no
16	M/61	WT	Parotid	46,XY,t(14;18)(q32;q21)[2]/46,X,−Y[3]/46,XY[6]	nt	nt	no
17	F/46	WT	Parotid	46,XX,t(5;8)(p15;q22)[2]/46,XX[10]	nt	nt	no

nt, not tested; nm, no metaphases; nr, no results.
*, Previously published as WT.[26]
†, Previously published.[26]

Materials and Methods

Case Selection

Case selection was based on the availability of karyotypic information and/or frozen tissue from the surgical specimen. All cases with the diagnosis of mucoepidermoid carcinoma and Warthin's tumor were reclassified according to the World Health Organization's criteria,[18] and MEC cases were graded according to Batsakis and Luna's criteria.[12,19] The clinical, histopathological, and karyotypic data are shown in Table 1.

Conventional Cytogenetic Analysis

Chromosome metaphases of tumor cells were obtained from short-term primary cultures as previously described.[20] Chromosomes were GTG-banded, and the karyotypes were defined according to International System of Human Cytogenetic Nomenclature (ISCN).[21] A detailed description of the tumor karyotypes is reported in Table 1.

ISH Analysis

ISH analysis was performed on cell suspension (metaphase and/or interphase nuclei) left from conventional cytogenetic analysis and on 4-μm formalin-fixed, paraffin-embedded tumor tissue sections. For the cell suspension, a standard ISH protocol with fluorescence detection (FISH) was applied.[22] For the tumor tissue sections, the ISH analysis followed the method of Alers et al,[23] using either fluorescence (FISH) or chromogenic (CISH) detection.

Two BAC clones probes named RP11–16K5 and RP11–676L3 that, together, cover the entire chromosome region of *MAML2* gene as described by Tonon et al[15] were used. The BAC clones DNA was isolated following the protocol of the distributor (Children's Hospital Oakland Research Institute, Oakland, CA, USA). The probes were labeled by random octamer priming (Bioprime DNA Labeling, Invitrogen SA, Barcelona, Spain). For FISH procedures, the BAC clones were labeled differently with biotin and digoxigenin and detected, respectively, by Cy3-avidin (Jackson Immunoresearch Lab, West Grove, PA, USA) and anti-digoxigenin-FITC (Roche Diagnostics GmbH, Mannheim, Germany). For CISH, both BAC clones were labeled with fluorescein (FITC) using horseradish peroxidase anti-fluorescein (Roche Diagnostics GmbH) and H_2O_2-diaminobenzidine for visualization.

In FISH preparations, the metaphases and nuclei material were counterstained with DAPI-Vectashield mounting solution (Vector, Burlingame, CA, USA). The CISH tissue sections were counter-stained with Meyer's hematoxylin and mounted with Entellan (Merck, Whitehouse Station, NJ, USA). The FISH and CISH signals were analyzed and recorded using a Cytovision System (Applied Imaging, Newcastle-on-Tyne, UK).

In each case, at least 200 interphase nuclei from paraffin-embedded tumor sections were evaluated. When present, normal salivary gland tissue was also analyzed. The presence of three dots (CISH preparations) or of split signals from BAC probes (FISH preparations) in more than 10% of the nuclei represent rearrangement of *MAML2* gene, following the criteria of Jenkins et al.[24]

RT-PCR Analysis

mRNA was extracted from tissue that was frozen in liquid nitrogen immediately after surgery, using the QuickPrep Micro mRNA purification Kit (Amersham Pharmacia Bio-

tech, Piscataway, NJ, USA), according to the manufacturer's protocol. As controls, one case of polymorphous low-grade adenocarcinoma (PLGA), one case of acinic cell carcinoma (ACC), and tissue from one normal parotid gland were used. Synthesis of cDNA was performed with approximately 0.3 μg of mRNA, at 37°C for 90 minutes, using random primers $p(dN)_6$ (Roche Diagnostics GmbH) and reverse transcriptase (SuperScript II RNase H^-, Invitrogen SA), following the manufacturer's instructions. Amplifications by PCR were carried out using 5 μl of cDNA, forward primer (5′-atggcgacttcgaacaatccgcggaa-3′) and reverse primer (5′-aacaggccattgccaggagaatgtgtatggg-3′). The oligonucleotide primers were designed to amplify a segment from the *MECT1-MAML2* cDNA, starting in exon 1 from the *MECT1* gene and finishing in exon 2 from the *MAML2* gene, with an expected size of 386 bp. One μl of each PCR reaction was then used as template for a second amplification using nested forward (5′-ggaaattcagcgagaagatcg-3′) and reverse (5′-tatgggatggcagagtgttagtc-3′) primers. These oligonucleotide primers amplify a segment of the *MECT1-MAML2* chimeric cDNA, starting in exon 1 from the *MECT1* gene and finishing in exon 2 from the *MAML2* gene, with an expected size of 339 bp. First round and nested RT-PCRs were performed over 35 cycles of amplification, using the following conditions: 95°C for 45 seconds, 56°C for 45 seconds, and 72°C for 1 minute. Amplification reactions, with a total volume of 50 μl, contained final concentrations of 20 mmol/L Tris-HCl (pH 8.4), 50 mmol/L KCl, 200 μmol/L dNTPs (Invitrogen SA), 1.5 mmol/L $MgCl_2$, 0.6 μmol/L of each primer, and 2U of *Taq*DNA polymerase (Invitrogen SA). Negative controls for cDNA synthesis and PCRs, in which the template was replaced by sterile water, were included in each experiment. RNA integrity and efficiency of cDNA synthesis were confirmed in each sample by RT-PCR for the housekeeping gene phosphoglycerate kinase 1 (*PGK1*), using previously published oligonucleotide primers.[25] Amplification with these primers yielded a 247-bp PCR product. PCR products were analyzed and purified by electrophoresis in a 2% agarose gel stained with ethidium bromide. All of the results were repeated, at least twice, from different batches of mRNA extracted at different times, from the same tumor. In the cases that presented the *MECT1-MAML2* rearrangement, the PCR product was sequenced (ABI Prism 310 Genetic Analyzer, using the ABI Prism Big Dye Terminator Cycle Sequencing Ready Reaction Kit version 2.0; Applied Biosystems, Foster City, USA).

Results

Cytogenetic Analysis

Cytogenetic information was obtained from all 17 cases except for case 9 due to unsuccessful cell culture and for case 2 which showed aneuploidy and abnormal metaphases, however a karyotype could not be defined due to unsatisfactory quality (Table 1). Five of the eight fully karyotyped MEC revealed a t(11;19)(q21;p13). This was the sole abnormality found in cases 4, 6, and 7 and the translocation was present together with other aberrations in cases 1 and 10. Figure 1a illustrates the usual histological features of MEC (case 6) and Figure 1b depicts the respective karyotype containing the t(11;19)(q21;p13) as the sole karyotypic aberration. Case 10 carried a more complex translocation t(11;16;19) that involved 16p13.3 in addition to 11q21 and 19q13. This case had been initially diagnosed as a WT,[26] and was reclassified as a MEC ex-WT (Figure 2a, case 10). Figure 2b illustrates the karyotype of case 10. Case 5 demonstrated two small clones with either loss of Y or gain of X and the remaining two MEC cases 3 and 8 had a normal karyotype. The series of WT contained three cases (12, 13, and 15) with normal karyotype and four with several abnormalities. None involved rearrangements of chromosome 11 and 19.

ISH Analysis

FISH Analysis on Cell Suspension

The five MEC cases with t(11;19) and case 2 were probed with the BAC clones probes RP11–16K5 and RP11–676L3 using FISH (Table 1). Rearrangement of *MAML2* gene in the t(11;19) positive cases was confirmed by a split signal from the two BAC clones. On metaphases, the signal from clone RP11–16K5, which contains the exon 1 of *MAML2* gene[15] was detected on der19 and the signal from clone RP11–676L3 on der11 (Figure 1c). In case 10, the signals from *MAML2* BAC clones were found on der11 and der16 (Figure 2c). Case 2, without karyotypic evidence of 11q21 and 19p13 rearrangements, showed no split signal.

ISH Analysis on Paraffin Sections

ISH analysis was performed in paraffin tumor tissue sections to verify *MAML2* rearrangements in uncultured tumor cells, *in situ*, and to compare the results obtained with cytogenetic and RT-PCR analysis. ISH was applied to seven MEC cases: this included all of the t(11;19) G-band positive cases, as well as case 3 with a normal karyotype and case 9 which could not be karyotyped (Table 1). Except for cases 4 and 10, where the signals could not be evaluated due to poor hybridization, all remaining cases confirmed *MAML2* rearrangement shown in FISH preparations by a split signal from BAC probes (Figure 1d) and in CISH by three brown dots (Figure 1e) in more than 10% of the cells. No *MAML2* rearrangement was observed in normal tissue (Figure 1f).

RT-PCR Analysis

To investigate the expression of the *MECT1-MAML2* chimeric gene, we carried out RT-PCR using tumor mRNA (Table 1). This analysis revealed the expression of the rearrangement between *MECT1* and *MAML2* genes in seven of the 10 (70%) mucoepidermoid carcinomas, but in none of the seven WT (Figure 3). Sequencing analysis

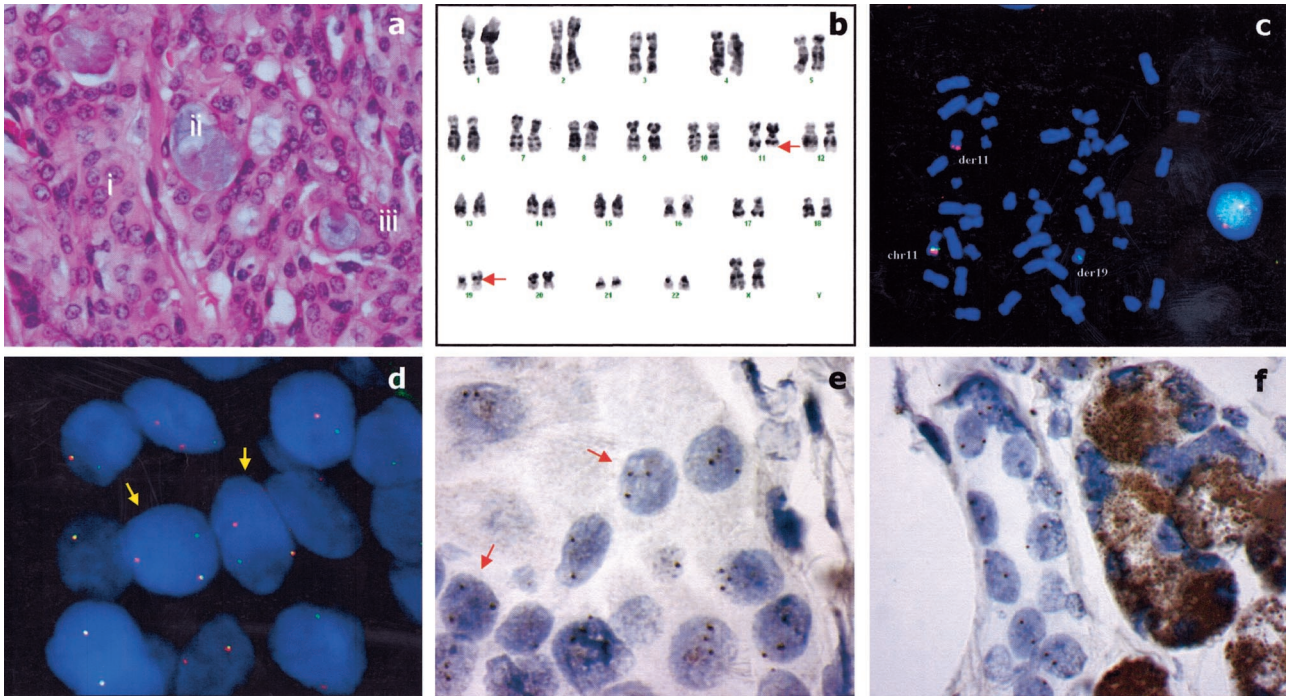

Figure 1. Mucoepidermoid carcinoma case 6 with a t(11;19)(q21;p13). **a:** Case 6 is a typical MEC, with mucin producing cells (ii), intermediate (iii), and squamous (i) cells (H&E). **b:** Representative karyotype showing the t(11;19) as the sole karyotypic abnormality. **c:** FISH analysis on a metaphase spread with the BAC clones probes RP11–16K5 (green) and RP11–676L3 (red) for *MAML2* gene showing the intragenic rearrangement of the gene: the hybridization probes are either located immediately adjacent in normal chromosome 11, or separated, one (green) from RP11–16k5 on der19 and the other (red) from RP11–676L3 on der11. **d:** FISH analysis on a paraffin tumor section with the BAC probes RP11–16K5 (red) and RP11–676L3 (green) for *MAML2* gene showing the intragenic rearrangement of the gene in interphase nuclei: the hybridization signals from both BAC probes are either located in close proximity indicating the normal gene *MAML2* or separated indicating rearrangement of the gene. **e:** CISH analysis of a tumor section from paraffin-embedded tissue. Both BAC clones probes RP11–16K5 and RP11–676L3 for *MAML2* gene were labeled with FITC and detected with DAB-chromogen (brown). There is an intragenic rearrangement of the gene in interphase nuclei: three brown spots could be observed representing one of them the normal copy of *MAML2* gene and two smaller signals the split of the gene. **f:** CISH analysis of normal salivary gland tissue, of the same section (**e**), using the same BAC probes, showing two spots per nucleus.

of the positive RT-PCR products confirmed the presence of the same rearrangement in the seven MECs, with the fusion product between *MECT1* exon 1 and *MAML2* exon 2. There was no expression of this rearrangement either in the two salivary glands carcinomas (ACC and PLGA) or in the normal salivary gland used as controls (Figure 3). We also carried out nested RT-PCR, which confirmed the results obtained in the first PCR (data not shown). The 247-bp RT-PCR product for the *PGK1* housekeeping gene was detected in all 20 samples, indicating adequate sampling and confirming mRNA integrity (Figure 3).

Discussion

A chromosomal translocation may be considered characteristic for a given cancer if it is detected frequently and, most importantly, if it occurs as the sole cytogenetic abnormality. The t(11;19)(q21;p12–13) chromosomal

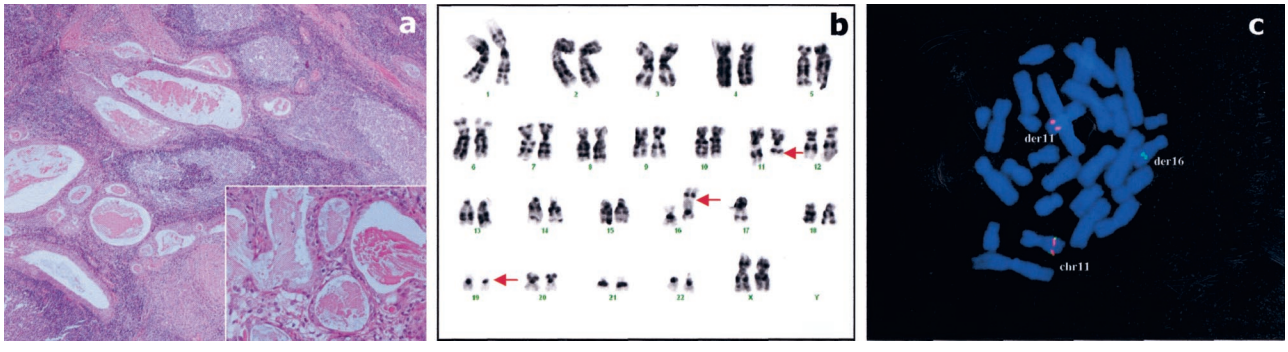

Figure 2. Mucoepidermoid carcinoma case 10 with a t(11;16;19)(q21;p13.3;p13). **a:** In low-power view the tumor presents cystic spaces, lined by eosinophylic cells and has a prominent lymphoid infiltration with follicules with germinal centers. Higher power demonstrates that the lining of the cysts is composed of the three cell populations that are diagnostic of MEC (H&E). **b:** Representative karyotype showing the t(11;16;19)(q21;p13.3;p13). **c:** FISH analysis on a metaphase spread with the BAC clones RP11–16K5 (green) and RP11–676L38 (red) for *MAML2* gene showing the intragenic rearrangement of the gene: both hybridization signals are immediately adjacent in normal chromosome 11, the hybridization signal from RP11–16k5 on der16 and the hybridization signal from RP11–676L3 on der11.

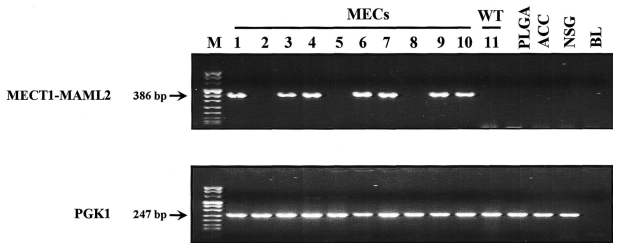

Figure 3. First round RT-PCR analysis of *MECT1-MAML2* fusion gene transcript, using *MECT1* exon 1 (sense) and *MAML2* exon 2 (antisense) oligonucleotide primers. RT-PCR products were resolved by electrophoresis on a 2% ethidium bromide-stained agarose gel and visualized on an UV source. MECs are numbered according to Table 1. *MECT1-MAML2* transcript (386-bp) is present in 7 of the 10 MECs, but not in seven Warthin's tumors (data shown only for Warthin's tumor 11), one PLGA, one ACC and one normal salivary gland (NSG). Replacing template with water (indicated as BL) yielded no product for both *MECT1-MAML2* and *PGK1* RT-PCRs. The detection of a 247-bp *PGK1* RT-PCR product confirmed mRNA integrity in all of the samples. **Lane 1:** DNA molecular weight marker VIII (Roche Diagnostics GmbH).

translocation meets these criteria as it is the most frequently detected aberration (27%) in MEC, either as the unique abnormality or together with other chromosome abnormalities.[3–9] The molecular cloning of the t(11;19)(q21;p13) translocation in two cell lines generated from lung and parotid gland MEC[15] has now allowed for specific RT-PCR and ISH to complement standard cytogenetics in the genetic characterization of malignant salivary gland tumors.

To evaluate the incidence of *MECT1-MAML2* fusion gene, we selected a series of 10 primary MEC which were cytogenetically characterized and then analyzed by ISH and RT-PCR. Additionally, seven WT cases were included in this study because a similar translocation t(11;19)(q13–21;p12–13) has been previously described in some of these tumors.[10,11] In addition, Enlund et al[27] reported that one case classified as WT and displaying a t(11;19) expressed the *MECT1-MAML2* fusion gene. WT and MEC are two otherwise unrelated salivary gland tumors that do not share clinicopathological features, and their accepted histogenesis is also distinct.[28] However, there are a few reports on the co-existence of both histological types in the same gland lesion,[13,14] which might be an explanation for the finding of a common genetic alterations. Case 10 (reported herein) is an example of such a situation. In addition, Case 1 represents an otherwise typical MEC but it also contains foci with a WT-like pattern.

All cases classified as MEC were evaluated by RT-PCR analysis to investigate the presence of expression of the fusion gene, using frozen tumor tissue. RT-PCR analysis detected *MECT1-MAML2* in all cases carrying the t(11;19) together with two other cases (one with normal karyotype, and one with an unsuccessful karyotyping). The expression of the chimeric gene in the case showing a normal karyotype is not unexpected. Normal karyotypes are known to occur in neoplasms due to contamination and/or overgrowth of normal elements such as lymphocytes and fibroblasts, or due to the insufficient resolution of conventional cytogenetics to recognize submicroscopic alterations. RT-PCR detection of the chimeric gene, therefore, proved to be a more sensitive approach for the diagnosis of genetic lesions in MEC than cytogenetic analysis. With the former method, we detected *MECT1-MALM2* fusion gene in 70% of the cases, as compared to 50% identified by conventional cytogenetics. In the appropriate clinical and histological setting, this leads to a potential usefulness of *MECT1-MALM2* as a molecular marker to make the diagnosis of mucoepidermoid carcinoma. In contrast, we did not detect the t(11;19) nor *MECT1-MALM2* expression in any of the seven primary WT samples.

RT-PCR, however, is hampered by the necessity to obtain suitable undegraded RNA from primary biopsy samples which may not be feasible in many situations. To circumvent this problem, we applied ISH analysis to paraffin-embedded tumor tissue sections to verify the presence of *MAML2* gene rearrangement in positive RT-PCR cases. The ISH technique performed on paraffin sections allows for the evaluation of the genetic alterations *in situ*, avoiding *in vitro* manipulation and the need for fresh tissue. In tumor sections, the *MAML2* rearrangement was successfully detected in five out of seven cases: in three t(11;19) G-band positive cases, in one normal G-band case, and in one unkaryotyped case which confirmed our RT-PCR results. In the remaining two cases, both t(11;19) G-band positive, no signal was obtained with the ISH analysis which was due to either poor fixation and/or preservation of the tissue fragments. Although the RT-PCR methodology appears to be the most sensitive technique for detecting *MECT1-MAML2*, the ISH approach is a reliable method that may allow the molecular diagnosis in extended case series where archival material can be used. Future studies using a larger series will ultimately define the proper role of RT-PCR and ISH for the diagnosis of MEC.

In cases 2, 5, and 8 there was no expression of the *MECT1-MAML2* fusion gene. None of these cases had a detectable t(11;19). The absence of *MECT1-MAML2* in 3 of 10 MEC samples warrants additional investigation and suggests an alternate genetic basis for this subset of salivary gland tumors. The cytogenetic information from published MEC case series show other recurrent chromosome aberrations, such as rearrangements affecting the chromosome region 6q21–25[29–32] and numerical abnormalities, such as loss of chromosome Y, and gains of chromosomes 2, 3, 5, 7, 18, 20, and X.[5,6,29,31–36] Some of these alterations are detected with the t(11;19) which might indicate they are secondary changes. However, some of them, such as the 6q rearrangements, are not associated with the t(11;19) chromosomal translocation. The role of these recurrent changes in the pathways of MEC carcinogenesis needs further clarification.

In conclusion, our data confirms and significantly extends the number of cases that have been analyzed to date, demonstrating a key role for the *MECT1-MAML2* fusion gene on MEC biology and also supporting the usefulness of ISH and RT-PCR for diagnosis, especially in histologically challenging cases. In contrast, *MECT1-MAML2* expression appears to be a rare event in WT samples that do not contain concomitant evidence for MEC.

Acknowledgments

We thank Lúcia Roque, B.Sc., Ph.D. and Valeriano Leite, M.D., Ph.D. for scientific advice and critical reading of the manuscript.

References

1. Ellis GL, Auclair PL: Tumors of the salivary glands. Atlas of Tumor Pathology. Edited by Rosai J, Sobin LH. Washington DC, Armed Forces Institute of Pathology, 1996, p 17
2. Mitelman F, Johansson B, Mertens F: Mitelman Database of Chromosome Aberrations in Cancer. http://cgap.nci.nih.gov/Chromosomes/Mitelman.2003
3. Dahlenfors R, Lindahl L, Mark J: A fourth minor salivary gland mucoepidermoid carcinoma with 11q14–21 and 19p12 rearrangements. Hereditas 1994, 120:287–288
4. Dahlenfors R, Wedell B, Rundcrantz H, Mark J: Translocation (11;19)(q14–21;p12) in a parotid mucoepidermoid carcinoma of a child. Cancer Genet Cytogenet 1995, 79:188
5. Hrynchack M, White V, Berean K, Horsman D: Cytogenetic findings in seven lachrymal gland neoplasms. Cancer Genet Cytogenet 1994, 75:133–138
6. Nordkvist A, Gustafsson H, Juberg-Ode M, Stenman G: Recurrent rearrangements of 11q14–22 in mucoepidermoid carcinoma. Cancer Genet Cytogenet 1994, 74:77–83
7. El-Naggar AK, Lovell M, Killary A, Clayman G, Batsakis J: A mucoepidermoid carcinoma of the minor salivary gland with t(11;19)(q21;p13.1) as the sole karyotypic abnormality. Cancer Genet Cytogenet 1996, 87:29–33
8. Johansson M, Mandahl N, Johansson L, Hambraeus G, Mitelman F, Heim S: Translocation 11;19 in a mucoepidermoid tumor of the lung. Cancer Genet Cytogenet 1995, 80:85–86
9. Stenman G, Petursdottir V, Mellgren G, Mark J: A child with a t(11;19)(q14–21;p12) in a pulmonary mucoepidermoid carcinoma. Virchows Arch 1998, 433:579–581
10. Bullerdiek J, Haubrich J, Meyer K, Bartnizke S: Translocation t(11;19)(q21;p13.1) as the sole chromosome abnormality in a cystadenolymphoma (Warthin's tumor) of the parotid gland. Cancer Genet Cytogenet 1988, 35:129–132
11. Mark J, Dahlenfors R, Stenman G, Nordquist A: A human adenolymphoma showing the chromosomal aberrations del(7)(p12;p14–15) and t(11;19)(q21;p12–13). Anticancer Res 1989, 9:1565–1566
12. Luna MA: Salivary mucoepidermoid carcinoma: revisited. Proceedings of the Joint Meeting of the Working Group on Head and Neck Pathology of the European Society of Pathology and the North American Society of Head and Neck Pathology of the United States and Canadian Society of Pathology. Ed. Gale N, Luzar B. Ljubljana, 2003, pp 99–105
13. Seifert G: Mucoepidermoid carcinoma in a salivary duct cyst of the parotid gland: contribution to the development of tumours in salivary gland cysts. Pathol Res Pract 1996, 192:1211–1217
14. Williamson JD, Simmons BH, El-Naggar AK, Medeiros J: Mucoepidermoid carcinoma involving Warthin tumor. Am J Clin Pathol 2000, 114:564–570
15. Tonon G, Mody S, Wu L, Kubo A, Coxon AB, Komiya T, O'Neil K, Stover K, El-Naggar AK, Griffin JD, Kirsch IR, Kaye FJ: t(11;19)(q21;p13) translocation in mucoepidermoid carcinoma creates a novel fusion product that disrupts a Notch signaling pathway. Nat Genet 2003, 33:208–213
16. Conkright MD, Canettieri G, Screaton R, Guzman E, Miraglia L, Hogenesch JB, Montminy M: TORCs: transducers of regulated CREB activity. Mol Cell 2003, 12:413–423
17. Iourgenko V, Zhang W, Mickanin C, Daly I, Jiang C, Hexham JM, Orth AP, Miraglia L, Meltzer J, Garza D, Chirn GW, McWhinnie E, Cohen D, Skelton J, Terry R, Yu Y, Bodian D, Buxton FP, Zhu J, Song C, Labow MA: Identification of a family of cAMP response element-binding protein co-activators by genome-scale functional analysis in mammalian cells. Proc Natl Acad Sci USA 2003, 100:12147–12152
18. Seifert G, Sobin LH. Histological typing of salivary gland tumours. World Health Organization International Histological Classification of Tumours, ed 2. New York, Springer-Verlag, 1991
19. Batsakis JG, Luna MA: Histopathologic grading of salivary gland neoplasms: i. Mucoepidermoid carcinoma. Ann Otol Rhinol Laryngol 1990, 99:835–838
20. Jin Y, Mertens F, Jin C: Non-random chromosome abnormalities in short-term cultured primary squamous cell carcinomas of the head and neck. Cancer Res 1995, 55:3204–3210
21. ISCN: An International System for Human Cytogenetic Nomenclature. Edited by Mitelman F. Basel, Karger, 1995
22. Höglund M, Johansson B, Pedersen-Bjergaard J, Marynen P, Mitelman F: Molecular characterization of 12p abnormalities in hematologic malignancies: deletion of KIP1, rearrangement of TEL, and amplification of CCND2. Blood 1996, 87:324–330
23. Alers JC, Krijtenburg PJ, Vissers KJ, Krishnadath SK, Bosman FT, Van Dekken H: Interphase in situ hybridization to disaggregated and intact tissue specimens of prostatic adenocarcinomas. Histochem Cell Biol 1995, 104:479–486
24. Jenkins B, Quian J, Lee HK, Huang H, Hirasawa K, Bostwick DG, Proffitt J, Wilber K, Lieber MM, Liu W, Smith DI: A molecular cytogenetic analysis of 7q31 in prostate cancer. Cancer Res 1998, 58:759–766
25. Sugg SL, Zheng L, Rosen IB, Freeman JL, Ezzat S, Asa SL: ret/PTC-1, -2, and -3 oncogene rearrangements in human thyroid carcinomas: implications for metastatic potential? J Clin Endocrinol Metab 1996, 81:3360–3365
26. Martins C, Fonseca I, Roque L, Soares J: Cytogenetic characterization of Warthin's tumour. Oral Oncol Eur J Cancer 1997, 33:334–347
27. Enlund F, Behboudi A, Andrén Y, Öberg C, Lendahl U, Mark J, Stenman G: Altered Notch signaling resulting from expression of a WANTP1-MAML2 gene fusion in mucoepidermoid carcinomas and benign Warthin's tumors. Exp Cell Res 2004, 292:21–28
28. Seifert G, Brocheriou C, Cardesa A, Eveson JW: WHO International Histological Classification of Tumors: tentative histological classification of salivary gland tumors. Pathol Res Pract 1990, 186:555–581
29. Sandros J, Mark J, Happonen R, Stenman G: Specificity of 6q-markers and other recurrent deviations in human salivary gland tumors. Anticancer Res 1988, 8:637–644
30. Jin Y, Mertens F, Limon J, Mandhal N, Wennerberg J, Dictor M, Heim S, Mitelman F: Characteristic karyotypic features in lachrymal and salivary gland carcinomas. Br J Cancer 1994, 70:42–47
31. Jin C, Martins C, Jin Y, Wiegant B, Wennerberg J, Dictor M, D. Gisselsson, B. Strombeck F: Mitelman, Tanke H, Fonseca I, Höglund M, Mertens F: Characterization of chromosome aberrations in salivary gland tumors by fluorescence in situ hybridization (FISH), including multicolor COBRA-FISH. Genes Chromosomes Cancer 2001, 30:161–167
32. Martins C, Fonseca I, Roque L, Pinto AE, Soares J: Malignant salivary gland neoplasms: a cytogenetic study of 19 cases. Oral Oncol Eur J Cancer 1996, 32B:128–132
33. Horsman DE, Berean K, Durham JS: Translocation t(11;19)(q21;p13.1) in a mucoepidermoid carcinoma of salivary gland. Cancer Genet Cytogenet 1995, 80:165–166
34. Nordkvist A, Staffan E, Mark J, Stenman G: Multiple unrelated chromosome abnormalities in a metastatic mucoepidermoid carcinoma of the parotid gland. Cancer Genet Cytogenet 1992, 61:158–161
35. El-Naggar AK, Lovell M, Killary A, Batsakis J: Trisomy 5 as the sole chromosomal abnormality in a primary mucoepidermoid carcinoma of the minor salivary gland. Cancer Genet Cytogenet 1994, 76:96–99
36. El-Naggar AK, Lovell M, Killary A, Batsakis J: Genotypic characterization of a primary mucoepidermoid carcinoma of the parotid gland by cytogenetic, fluorescence in situ hybridization, and DNA ploidy analysis. Cancer Genet Cytogenet 1996, 89:38–43